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(54) **Method for preparing zinc-oligopeptide easily absorbable by the human body**

(57) Disclosed is a method for preparing zinc-oligopeptides easily absorbable by the human body. A suspension of protein in deionized water at a neutral pH range in the presence of a protease is subjected to proteolysis to give a mixture of oligopeptides. Zinc ions are chelated with the oligopeptides to give a zinc-oligopeptide solution. The zinc-oligopeptide solution is concentrated and dried to a powder. Also provided is a beverage or food composition containing the zinc-oligopeptide, which can make contribution to avoid the lack of dietary zinc.

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Description

[0001] The present invention relates to a method for preparing zinc-oligopeptides which can be easily absorbed by the human body. Also, the present invention is concerned with a beverage or food composition, which can easily provide zinc for the human body.

[0002] Loss of the mineral balance in the body has been found as one of the causes of adult diseases, which have recently been increasing in incidence. With growing older, the human body becomes poorer at absorbing minerals. Nowadays, a deluge of processed foods is becoming as one main cause of upsetting the mineral balance of the body because most of them contain materials inhibiting mineral absorption.

[0003] Of various minerals, zinc is involved particularly in the onset of diabetes mellitus, one of the most popular diseases today. In fact, not only adults, but also a surprising number of children suffer from the disease at present, which is believed to be attributed in part to the lack of zinc. In Oriental medicine, zinc is also described to have physiological activity associated with sugar control and vigor in the body. Deeply affected in diabetes mellitus patients, the blood sugar control of the body has direct influence on the energy production necessary for life. Although other physiological functions are active, abnormal regulation of blood sugar in the body, which means that the metabolic process of converting sugar into energy is in an abnormal state, lowers the immunity of the body to exogenous pathogens as well as latent viruses, resulting in the body falling ill.

[0004] In the body, zinc serves as an essential mineral in activating insulin, in addition to being involved in regenerating muscle tissues and nerve tissues. Abundant as it is in blood, insulin cannot exert its full effects in the absence of zinc. That is, insulin which is not associated with zinc is not beneficial to diabetics. A similar case can be found with amylase, which is unable to function as a saccharification catalyst unless it is associated with ionic calcium.

[0005] One of the most important physiological activities of zinc is to activate insulin into a form useful in the treatment of diabetes mellitus. In turn, the activated insulin is responsible, in great part, for the production of energy. In addition, zinc was found to inhibit the expression of mutant genes, thereby making a contribution to anticancer activity. Further, zinc is known to take part in a catalytic reaction necessary for DNA polymerization and therefore affect the rapid regeneration of injured tissue. In this regard, zinc has some connection with acceleration of wound healing, prevention of prostate problems and hair loss, and treatment of acne and rheumatoid arthritis.

[0006] Recently, sufficient intake of zinc has been reported to significantly decrease the incidence of disease in children. When their diets are supplemented with zinc, children are 40 % less likely to be taken ill with pneumonia and 25 % less likely to get diarrhea. As stated above, diabetes mellitus may be caused when dietary zinc is insufficient. When insulin is not activated, the body is significantly deprived of available sugar, leading to loss of vigor.

[0007] In order to be activated in association with zinc, the insulin must not be in a pro-insulin form, but in a functional form. Stoichiometrically, one molecule of functional insulin (molecular weight 6,615) associates with a zinc atom (atomic weight 65). Therefore, functional insulin must be associated with zinc at a weight ratio of approximately 1,000:1. In the medical world, globulin zinc Insulin is used as an insulin formulation, which is generally for subcutaneous injection, for the purpose of zinc activation of insulin.

[0008] Occurrence of diabetes mellitus in children in recent times, which was rare in the past, is believed to be strongly affected by dietary lifestyles, but not heredity. Fried foods, which children usually like, contain a large quantity of fat that suppresses the activity of zinc. Also, a high intake of processed foods inhibits activities of calcium as well as zinc because of their high contents of phosphoric acid. Lipids and phosphates are known to actively inhibit the absorption of minerals irrespective of which form they have.

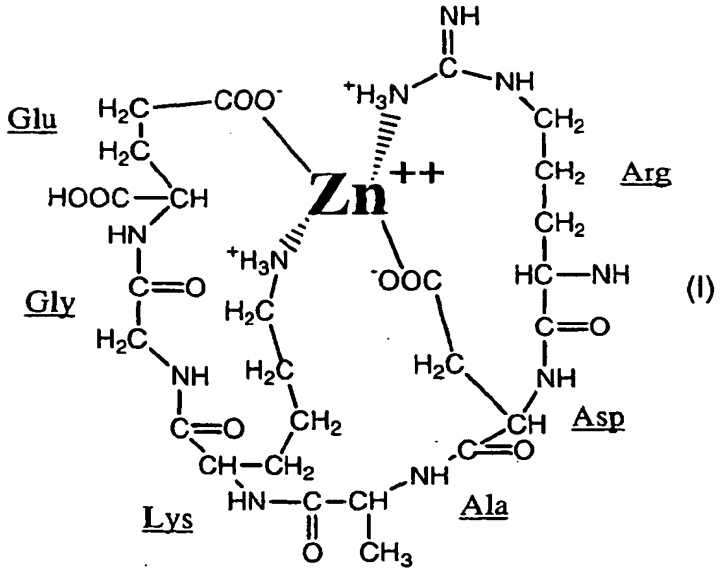
[0009] Naturally, living bodies have to supplement consumed or deficient materials by themselves. Hence, it is necessary that people exercise restraint in their ingestion of materials which inhibit such a natural supplementary function. The loss of the supplementary functions owing to ingestion of inhibitory materials causes a vicious cycle of deficiency. For instance, deficient dietary zinc lowers the activity of insulin, and unconsumed insulin causes a decrease or ceasing of the production of insulin in the pancreas. On the other hand, when insulin is actively consumed, it must be replaced, and thus insulin is more actively produced in the pancreas. This is demonstrated by the fact that athletes, who consume large energy, contain zinc at an amount 20 times as much as that of ordinary people. Zinc enables insulin to actively promote the metabolism of sugar in the body.

[0010] However, most of the mineral-enriched materials developed thus far, are poor in absorbability by the human body. Calmodulin, a calcium-chelated oligopeptide, was found in neurotic system of the human body, and to be absorbed easy as Calmodlin molecular structure by the small intestine.

[0011] Hinted by the structure of calmodulin, the intensive and thorough research on facilitating of the human body to absorb zinc, conducted by the present inventors, resulted in the finding that minerals are better absorbed by the body when they combine with organic materials, especially oligopeptides, rather than alone.

[0012] Therefore, it is an object of the present invention to provide a method for preparing a zinc-oligopeptide which can be easily absorbed by the body.

[0013] Accordingly the invention concerns a compound according to formula I



(I)

wherein Glu = glutamic acid; Asp= aspartic acid; Lys= lysine; Arg= arginine; Gly= glycine and Ala= alanine.

[0014] It is another object of the invention to provide a method for preparing a zinc-oligopeptide which can be easily absorbed by the body.

[0015] It is another object of the present invention to provide use of zinc-oligopeptide in foods.

[0016] In accordance with an aspect of the present invention, there is provided a method for preparing a zinc-oligopeptide easily absorbable by the body, comprising the steps of: proteolyzing a suspension of protein in deionized water at a neutral pH range in the presence of a protease to give a mixture of oligopeptides; chelating zinc ions with the oligopeptides to give a zinc-oligopeptide solution; concentrating the zinc-oligopeptide solution and drying the concentrate to a powder.

[0017] In accordance with another aspect of the present invention, there is provided a beverage comprising the zinc-oligopeptide, in combination with at least one ingredient selected from the group consisting of vitamin-C, vitamin-B_1, vitamin-B_2, fructose, α-amylase decomposed starch and magnesium stearate.

Fig. 1 shows a structure of an oligopeptide associated with a zinc ion.

Fig. 2 shows a structure of an oligopeptide alone.

[0018] In accordance with one embodiment of the present invention, there is provided a method for preparing zinc-oligopeptide. First, a suspension of protein in deionized water is subjected to proteolysis, e.g. with enzymes such as pepsin, trypsin or protease, in a neutral pH range from 3.5 to 6.0, preferably 6.8 to 9.0, for a long period of time to give oligopeptides. These are used to chelate zinc ions to form zinc-oligopeptides. The resulting solution is concentrated and dried to yield zinc-oligopeptide powder.

[0019] In detail, 100 weight parts of protein are suspended in 800 weight parts of deionized water and added with 2 to 4, preferably 3 to 4, weight parts of protease. Proteolytic reaction is conducted at pH 3.5-6.0, preferably 6.8 to 9.0, for 10 to 12 hours to give oligopeptides. Based on 1,000 weight parts of the oligopeptide thus obtained, one weight part of zinc ions is mixed and allowed to chelate, to yield a zinc-oligopeptide. Then, the resulting zinc-oligopeptide solution is concentrated to a solid content of 32 to 36 weight percent, preferably 32.5 to 36 weight percent, and dried to produce zinc-oligopeptide powder.

[0020] Either vegetable protein or animal protein may be used for obtaining oligopeptides.

[0021] In accordance with another embodiment of the present invention, there is provided a zinc-oligopeptide-containing beverage. To this end, vitamin-C, vitamin-B_1, vitamin-B_2, fructose, α-amylase decomposed starch, and/or magnesium stearate may be mixed with liquid zinc-oligopeptide. Alternatively, this composition is dehydrated to give powder suitable for use in capsules or tablets.

[0022] More specifically, 95.5 weight percent of the zinc-oligopeptide is mixed with 0.01 to 0.05 weight percent of vitamin-C, and/or 0.01 to 0.05 weight percent of vitamin-B_1, and/or 0.01 to 0.05 weight percent of vitamin-B_2, and/or 3.0 to 4.0 weight percent of α-amylase decomposed starch, and/or 0.01 to 0.05 weight percent of magnesium stearate.

[0023] The zinc-oligopeptide of the present invention has a molecular weight of from 800 to 1,200. Because its

molecular weight is smaller than the average molecular weight (24,000-28,000) of membrane integral proteins of the small intestine, through which molecules pass in and out of the cell, the zinc oligopeptide of the present invention can be readily absorbed by the body. Additionally, zinc is chelated by water-soluble oligopeptides, so that its absorption by the body is not inhibited by other compounds present in the digestive tract.

[0024] A better understanding of the present invention may be obtained in light of the following examples.

EXAMPLE 1

[0025] As an oligopeptide source, a vegetable protein such as bean protein and gluten, or an animal protein such as casein and gelatin was used. 100 weight parts of protein with a purity of 95 % or higher was suspended in 800 weight parts of water and allowed to undergo proteolysis in the presence of 3 weight parts of protease at pH 6.8 to 9.0 for 12 hours to give oligopeptides. Of the proteolysates, non-water soluble ones were filtered off. One gram atom of zinc (65.36 g) was added based on 1,000 weight parts of the oligopeptide to form a chelated zinc-oligopeptide composite. Then, the resulting solution was concentrated in a concentrator to a solid content of 35 weight percent and spray-dried to give a zinc-oligopeptide powder.

EXAMPLE 2

[0026] The zinc-oligopeptide solution prepared in Example 1 was controlled to have a concentration of 28 weight percent and formulated as indicated in Table 1, below, to provide a zinc-oligopeptide beverage with a full recommended dietary allowance (RDA) of zinc (12 to 15 mg/day).

TABLE 1

Composition of Zinc-Oligopeptide Beverage	
Ingredients	Amounts (wt%)
28% Zn-Oligopeptide Sol'n	1.0
Vitamin-B_1	0.01
Fructose	12
Vitamin-C	0.03
Vitamin-B_2	0.01
Water	86.95
Total	100

EXAMPLE 3

[0027] The zinc-oligopeptide prepared in Example 1 was formulated with other ingredients as shown in Table 2, below, and 0.5 g of the resulting mixture was filled into a capsule.

TABLE 2

Composition of Zinc-Enriched Capsule	
Ingredients	Amounts (wt%)
Zn-Oligopeptide Powder	99.95
Vitamin-B_1	0.01
Vitamin-C	0.03
Vitamin-B_2	0.01
Total	100

EXAMPLE 4

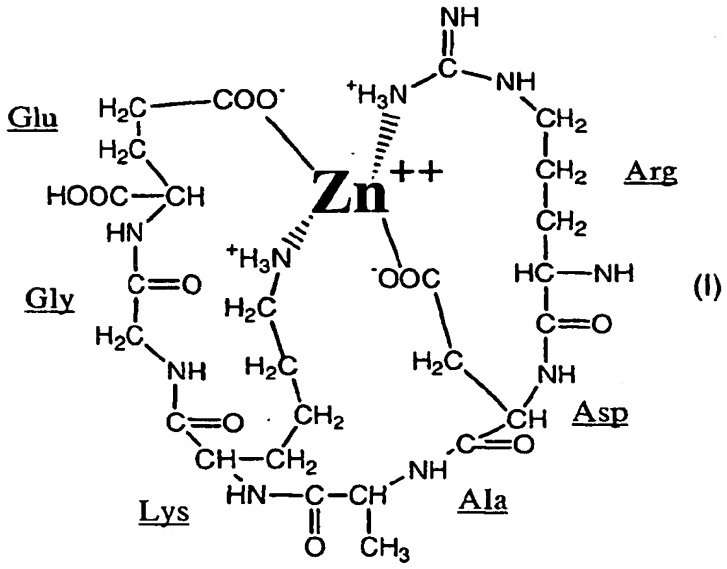
[0028] The zinc-oligopeptide prepared in Example 1 was formulated with other ingredients as shown in Table 3, below, and 0.5 g of the resulting mixture was formed into a zinc-enriched tablet.

TABLE 3

Composition of Zn-Enriched Tablet	
Ingredients	Amounts (wt%)
Zn-Oligopeptide Powder	94.95
Vitamin-B_1	0.01
α-Amylase Decomposed starch	4.7
Vitamin-C	0.03
Vitamin-B_2	0.01
Mg Stearate	0.3
Total	100

Claims

1. A compound according to formula I



wherein Glu = glutamic acid; Asp= aspartic acid; Lys= lysine; Arg= arginine; Gly= glycine and Ala= alanine.

2. The compound according to claim 1 which has a molecular of 800 to 1,200.

3. A method for preparing a zinc-oligopeptide, comprising the steps of:

proteolyzing a suspension of protein in water at pH 6.8 to 9.0 in the presence of a protease to give a mixture of oligopeptides; and
chelating zinc ions with the oligopeptides to give a zinc-oligopeptide solution.

4. The method of claim 3, further **characterized in that** the zinc-oligopeptide solution is concentrated and dried to a powder.

5. The method as claimed in claim 3 or 4, further **characterized in that** the water is deionized water.

6. The method as claimed in claims 3, 4 or 5, further **characterized in that** the protein is proteolyzed at a pH of 3.5

to 6.0.

7. The method as set forth in one of claims 3 to 6, wherein the protein is an animal protein or a vegetable protein.

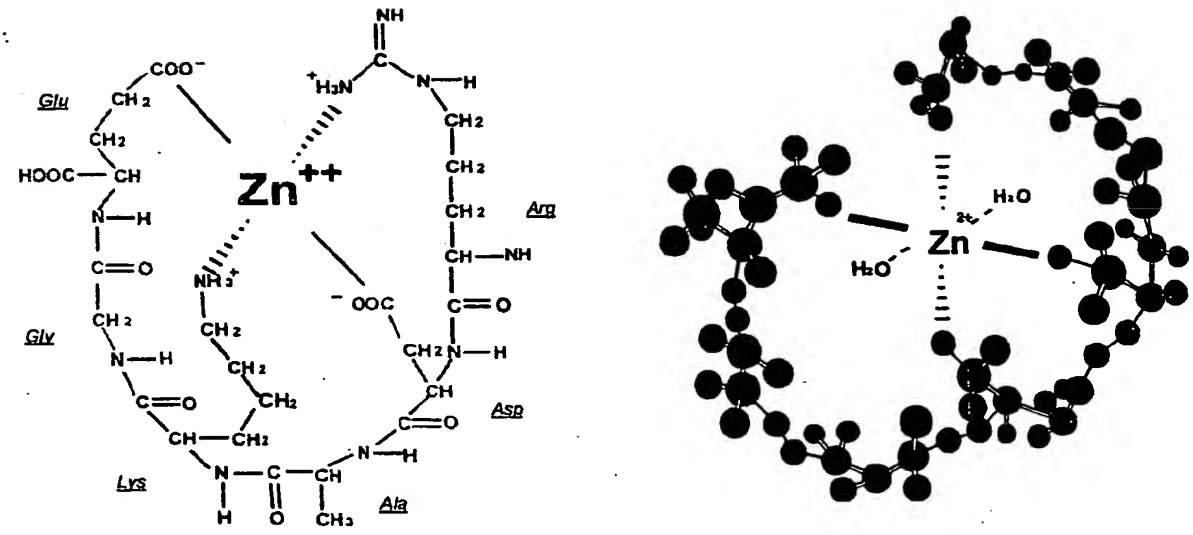
8. The method as set forth in claim 3, **characterized in that** 100 weight parts of protein is suspended in 800 weight parts of deionized water and proteolyzed at pH 3.5 to 6.0 for 10 to 12 hours in the presence of 2-4 weight parts of protease to give oligopeptides, one gram atom of zinc ions is mixed based on 1,000 weight parts of the oligopeptide and allowed to chelate, to yield a zinc-oligopeptide, and the resulting zinc-oligopeptide solution is concentrated to a solid content of 32 to 36 weight percent and dried to produce zinc-oligopeptide powder.

9. A beverage, comprising the zinc-oligopeptide of claim 1, in combination with at least one ingredient selected from the group consisting of vitamin-C, vitamin-B_1, vitamin-B_2, fructose, α-amylase decomposed starch and magnesium stearate.

10. The beverage as set forth in claim 9, wherein a composition containing 95.5 weight percent of the zinc-oligopeptide and 0.01 to 0.05 weight percent of vitamin-C, and/or 0.01 to 0.05 weight percent of vitamin-B_1, and/or 0.01 to 0.05 weight percent of vitamin-B_2, and/or 3.0 to 4.0 weight percent of α-amylase decomposed starch, and/or 0.01 to 0.05 weight percent of magnesium stearate (all weight percent based on the total weight of the composition) is added to the beverage.

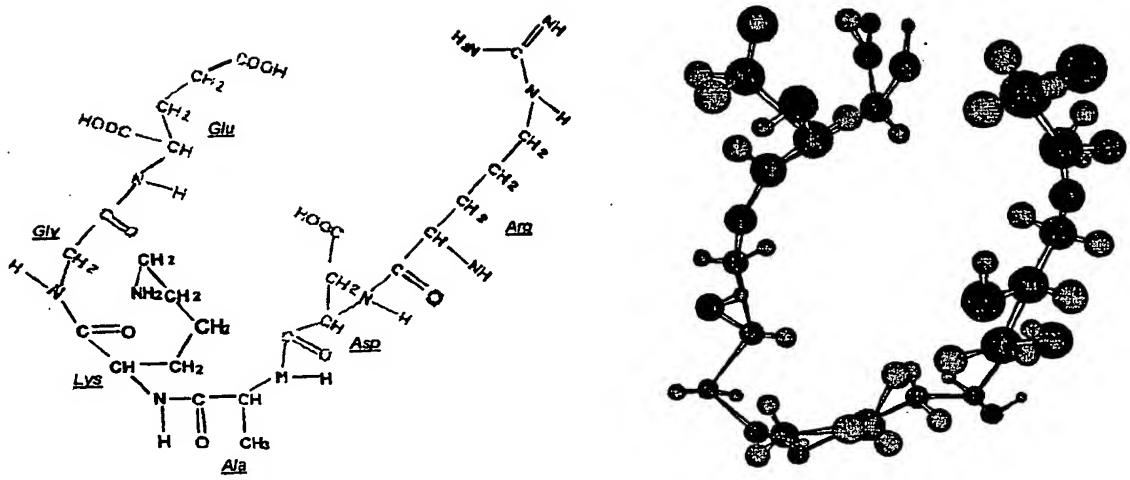
11. A capsule or tablet, prepared by dehydrating the zinc-oligopeptide beverage of claim 10.

FIG. 1



Zinc-Oligopeptide

FIG. 2



Oligopeptide

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(54) **Method for preparing zinc-oligopeptide easily absorbable by the human body**

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EUROPEAN SEARCH REPORT

Application Number
EP 01 71 0010

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
A	US 4 680 276 A (BRICAS EVANGHEIOS ET AL) 14 July 1987 (1987-07-14) * claims; example 3 * ---	1,2	C07K7/06 C07K14/415 C07K14/435 A23L1/304
A	CARPENTER KATHARINE A ET AL: "Aggregation behaviour and Zn2+ binding properties of secretin" BIOCHEMISTRY, AMERICAN CHEMICAL SOCIETY. EASTON, PA, US, vol. 37, no. 48, 1 December 1998 (1998-12-01), pages 16967-16974, XP002161821 ISSN: 0006-2960 * page 16968, right-hand column, last paragraph - page 16969, left-hand column, paragraph 1 * * page 16970, right-hand column, last paragraph - page 16971, left-hand column, paragraph 1 * ---	1,2	A23J3/34
A	US 4 412 988 A (GEOFFRE SERGE ET AL) 1 November 1983 (1983-11-01) * column 9, line 26 - column 10, line 10; claims * -----	1,2	TECHNICAL FIELDS SEARCHED (Int.Cl.7) C07K

~~The present search report has been drawn up for all claims~~

Place of search	Date of completion of the search	Examiner
THE HAGUE	13 November 2001	FUHR, C

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)

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LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 01 71 0010

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. Claims: 1 2 9-11

   complex of hexapeptide Arg-Asp-Ala-Lys-Gly-Glu with zinc; beverage, tablet or capsule compr. complex

2. Claims: 3-8

   method of preparation of zinc-oligopeptides by proteolysis;

The claims of the application relate to a plurality of inventions which present solutions for different problems, being:
a) Claims 1,2 and 9-11 disclose the provision of a complex of a specific hexapeptide with zinc and beverages and capsules and tables comprising it. The solution to this problem consists of a compound disclosed in the application.
b) Claims 3-8 disclose the provision a method for proteolysis of proteins and subsequent addition of zinc to form mixtures of oligopeptide-zinc complexes. The method solving this problem appears to be of a general nature, not specifically designed for the preparation of a compound encompassed by the application. Moreover the compound of the application can be prepared with usual methods well-known in the prior art.
Therefore these problems, each having a solution of their own, are not linked by a special technical feature that could unify them.
This plurality of solutions might, a priori, be considered as satisfying the requirements of unity in which the provision zinc complexing compounds provides the special technical feature linking these different solutions.
However at the first priority date of the application oligopeptides compexing zinc were already well-known in the prior art, as can be exemplified by US4680276 and US4412988 and K.A. Carpenter and P.W. Schiller " Aggregation Behaviour and Zn2+ Binding Properties of Secretin" in Biochemistry 37, 1988, pages 16967-16974.
In the light of these documents, the search division considers that a common technical link based on the zinc complexing properties of the oligopeptides of the application which could be the unifying concept is no longer present.

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 01 71 0010

This annex lists the patent family membersrelating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
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13-11-2001

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82